REMARKS

Status of the Claims

Claims 1-13 and 18-29 are currently pending in this application. In this amendment, claims 1 and 21 are amended to clarify the invention; claims 4, 5, 26, 27 and 29 are canceled without prejudice or disclaimer; and new claims 37 and 38 are added. Support for the amendment may be found throughout the specification as filed, for example, at page 12, lines 21-23, page 13, lines 19-23 and page 15, lines 10-15; in Examples 1 and 2; and in original claims 16, 26 and 27. Thus, no new matter has been added. Upon entry of the amendment, claims 1-3, 6-13, 18-25, 28, 37 and 38 will be subject to further examination. Entry of the amendment and reconsideration on the merits in view of the following comments is respectfully requested.

With respect to all amendments, Applicants have not dedicated or abandoned any unclaimed subject matter and have not acquiesced to any rejections and/or objections made by the Patent Office. Applicants expressly reserve the right to pursue prosecution of any presently excluded subject matter or claim embodiments in one or more future continuation and/or divisional application(s).

Correction of Inventorship

In reviewing their files, Applicants recently noticed that the name of one of the inventors was inadvertently misspelled in the parent international application PCT/CN2002/000940, in the Application Data Sheet filed on June 10, 2005 concurrently with the present application, and in the inventors' Declaration filed on October 11, 2005 in response to a Notice to File Missing Parts. Please note that the correct name of the inventor currently identified as **Jing CHEN** is **Jing CHENG**. In case a formal petition and/or a replacement Declaration must be filed to correct this typographical error, Applicants would greatly appreciate the Office's detailed instructions as to the most cost-efficient way to address this problem.

Rejections under 35 U.S.C. § 103

Dauer in View of Grevelding

Claims 1-4, 6-9, 12-13, 18, 20-25 and 28 are rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Dauer *et al.* (*Biotechnol. Bioeng.*, 37:1021-1028 (1991), hereinafter "Dauer") in view of Grevelding *et al.* (*Nucleic Acids Res.*, 24(20):4100-4101 (1996), hereinafter "Grevelding").

Dauer allegedly teaches a method of isolating cells using magnetic particles. With regard to claim 1, Dauer allegedly teaches a process for amplifying a nucleic acid of a target cell or virus, which process comprises: a) contacting a sample containing or suspected of containing a target cell or virus with a magnetic microbead not comprising a biomolecule that binds to said target cell or virus with high specificity (p. 1024, col. 2, where baker's yeast were the target cells and where the magnetic microbead comprises a magnetic "seed" comprising ferromagnetic gamma-iron oxide or maghemite (Fe_2O_3); see Table 1); b) allowing said target cell or virus, if present in said sample, to bind to said magnetic microbead to form a conjugate between said target cell or virus and said magnetic microbead (Figure 6, where the process of mixing, binding and separation are depicted; p. 1025. col. 2, and where the pH is used to control binding to the particles and then release of the particles); c) separating said conjugate from other undesirable constituents via a magnetic force to isolate said target cell or virus from said sample (Figure 6E, where the conjugate between the magnetic particle and the cells are separated from the sample), wherein said biomolecule is selected from the group consisting of an antibody, an amino acid, a peptide, a protein, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a vitamin, a monosaccharide, an oligosaccharide, a carbohydrate, a lipid and a complex thereof (Table 1, p. 1024, col. 2, where the magnetic particle is not coated with a biomolecule or other affinity group).

The Office acknowledges that Dauer does not explicitly teach that the cells can be applied to an amplification system. To cure this deficiency of Dauer, the Office cites Grevelding, which allegedly teaches a method comprising d) applying said separated conjugate to a nucleic acid amplification system to amplify a nucleic acid from said target cell or virus, wherein said process does not comprise a step of lysing said target cell or virus to release said nucleic acid prior to

applying said separated conjugate to said nucleic acid amplification system (Abstract, p. 4100, col. 1, where the technique of PCR is applied to whole organisms and has been applied to yeast and bacteria).

The Office argues that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have adjusted the method of Dauer to include further analysis of the captured and released cells using PCR amplification as taught by Grevelding to arrive at the claimed invention with a reasonable expectation for success. Grevelding states that "recently protocols were introduced that allow PCR amplification without DNA extraction" and "we show that PCR amplification is possible from whole, undissected larvae and adults of the fruitfly *Drosophila melanogaster* and the blood fluke, *Schistosoma mansoni* without preceding DNA isolation." Since Grevelding teaches isolation from whole organisms, the Office argues that the technique of amplification directly from cells without prior DNA extraction is supported by the teachings of Grevelding. Therefore, the Office concludes that one of ordinary skill in the art would have been motivated to have adjusted the method of Dauer to include further analysis of the captured and released cells using PCR amplification as taught by Grevelding to arrive at the claimed invention with a reasonable expectation for success.

Applicants respectfully traverse this rejection for the reasons set forth below.

As an initial matter, this rejection is rendered moot with respect to claims 2 and 3 by the cancellation of these claims. Additionally, claim 1 has been amended to specify that the target cell is a leukocyte or epithelial cell, and that the target cell, if present in the sample, binds to the magnetic microbead nonspecifically or with low specificity. As noted above, support for this amendment is found in the application as filed at least at page 12, lines 21-23, page 13, lines 19-23 and page 15, lines 10-15; in Examples 1 and 2; and in original claims 16, 26 and 27. Since each of claims 4, 6-9, 12-13, 18, 20-25 and 28 depends, directly or indirectly, from claim 1, all of these claims incorporate the new limitations of claim 1 as well.

The obviousness analysis under 35 U.S.C. § 103(a) requires the consideration of the scope and content of the prior art, the level of skill in the relevant art, and the differences between the prior art and the claimed subject matter must be considered. *KSR Int'l Co. v. Teleflex Inc.*, 127 S.Ct. 1727 (2007) (*citing Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966)). Rejections on obviousness grounds cannot be sustained by mere conclusory statements. *In re Kahn*, 441 F.3d 977, 987-88 (Fed. Cir. 2007) (citations omitted). Critical elements of the invention as a whole which clearly distinguish the entire invention from the prior art references cannot be ignored. *Panduit Corp. v. Dennison Manufacturing Co.*, 1 U.S.P.Q.2d 1593, 1597 (Fed. Cir.), *cert. denied*, 481 U.S. 1052 (1987). Evidence of an unobvious or unexpected advantageous property can rebut *prima facie* obviousness. MPEP § 716.02(a).

Dauer teaches the use of high gradient magnetic separation (HGMS) to separate nonmagnetic microorganisms such as the baker's yeast (*Saccharomyces cerevisiae*) from solution by a technique known as seeding, whereby fine magnetic particles are adhered to the cells' surfaces, making them magnetic and amenable to magnetic separation. (*See* Abstract). Dauer further teaches that technique may be used to recover microorganisms from dilute process streams and is particularly well suited to the final clean-up and isolation of proprietary or hazardous organisms. (*See* Dauer at page 1027, right col.) Thus, Dauer is primarily concerned with large-scale magnetic separation of microorganisms from solution.

Grevelding teaches that direct DNA amplification by PCR may be performed on fruitflies and blood flukes without DNA purification. Grevelding also teaches that similar protocols had been developed for microorganisms such as protozoans, bacteria and yeast. (*See* Grevelding at page 4100, left col.) Grevelding does not contain any discussion regarding magnetic separation.

Neither Dauer nor Grevelding teaches or even suggests nonspecific or low-specificity magnetic separation of mammalian cells, particularly leukocytes or epithelial cells. Moreover, neither Dauer nor Grevelding provides any indication that direct PCR amplification would be effective on mammalian cells, particularly leukocytes or epithelial cells. Since the combination of Dauer and Grevelding fails to teach several important elements of claim 1 as amended herein, the

Office has failed to establish a *prima facie* case of obviousness. Accordingly, it is respectfully submitted that this rejection under 35 U.S.C. § 103(a) may properly be withdrawn.

Dauer in View of Grevelding and Further in View of Lopez-Sabater

Claims 5 and 29 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Dauer in view of Grevelding as applied to claims 1-4, 6-9, 12-13, 18, 20-25 and 28 above, and further in view of Lopez-Sabater *et al.* (*Lett. Appl. Microbiol.*, 24:101-104 (1997), hereinafter "Lopez-Sabater").

This rejection is rendered moot by the cancellations of claims 5 and 29.

Dauer in View of Grevelding and Further in View of Ughelstad

Claim 10 is rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Dauer in view of Grevelding as applied to claims 1-4, 6-9, 12-13, 18, 20-25 and 28 and further in view of Ughelstad *et al.* (WO 83/103920 (1983), hereinafter "Ughelstad").

The Office acknowledges that Dauer does not explicitly teach that the magnetic beads can be made of Fe_3O_4. To cure this deficiency of Dauer, the Office cites Ughelstad, which allegedly teaches magnetic beads for use in separation wherein the metal composition is Fe_3O_4.

The Office argues that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have applied the specific teachings of Ughelstad to the particles of Dauer to arrive at the claimed invention with a reasonable expectation for success. Dauer allegedly states: "The magnetic seed is a ferromagnetic γ-iron oxide (γ-Fe_2O_3) or maghemite" (p. 1024, col. 2). Ughelstad teaches wherein the method composition comprises Fe_3O_4 specifically (see p. 9). Therefore, the Office argues that one of ordinary skill in the art at the time the invention was made would have been motivated to have applied the specific teachings of Ughelstad to the particles of Dauer to arrive at the claimed invention with a reasonable expectation for success.

As noted above, claim 1 has been amended to specify that the target cell is a leukocyte or epithelial cell, and that the target cell, if present in the sample, binds to the magnetic microbead nonspecifically or with low specificity. Since claim 10 indirectly depends from claim 1, claim 10 incorporates the new limitations of claim 1 as well.

The teachings of Dauer and Grevelding have been briefly discussed above. Ughelstad teaches various magnetic polymer particles prepared by treating compact or porous polymer particles with a solution of iron salts and, optionally, salts of other metals which are capable of forming magnetic ferrites, in which the solution swells or penetrates into the particles. (Abstract.)

Much like Dauer and Grevelding, Ughelstad does not teach or suggest nonspecific or low-specificity magnetic separation of mammalian cells, particularly leukocytes or epithelial cells. Moreover, much like Dauer and Grevelding, Ughelstad does not provide any indication that direct PCR amplification would be effective on mammalian cells, particularly leukocytes or epithelial cells. Since the combination of Dauer, Grevelding and Ughelstad fails to teach several important elements of claim 10 as amended herein, the Office has failed to establish a *prima facie* case of obviousness. Accordingly, it is respectfully submitted that this rejection under 35 U.S.C. § 103(a) may properly be withdrawn.

Dauer in View of Grevelding and Further in View of Dzieglewska

Claims 11, 19 and 27 are rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Dauer in view of Grevelding as applied to claims 1-4, 6-9, 12-13, 18, 20-25 and 28 and further in view of Dzieglewska (WO 98/51693 (1998), hereinafter "Dzieglewska").

The Office acknowledges that Dauer does not explicitly teach the additional limitations of claims 11, 19 and 27. To cure these deficiencies of Dauer, the Office cites Dzieglewska, which allegedly teaches magnetic microbead having a diameter ranging from about 5 to about 50,000 manometers (claim 11); an automated method of nucleic acid isolation (claim 19); and a target cell comprising a bacteria or eukaryotic cell and can be obtained from a urine sample).

The Office argues that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have adjusted the teachings of Dauer to the include elements of Dzieglewska to arrive at the claimed invention with a reasonable expectation for success. Although the Office acknowledges that Dzieglewska teaches a method comprising lysis of cells prior to amplification, the Office argues that the elements of the claims represented by Dzieglewska are obvious in combination with the teaching of Dauer and Grevelding. Accordingly, the Office concludes that one of ordinary skill in the art at the time the invention was made would have been motivated to have adjusted the teachings of Dauer and Grevelding to the include elements of Dzieglewska to arrive at the claimed invention with a reasonable expectation for success.

As an initial matter, this rejection is rendered moot with respect to claim 27 by the cancellation of this claim. As noted above, claim 1 has been amended to specify that the target cell is a leukocyte or epithelial cell, and that the target cell, if present in the sample, binds to the magnetic microbead nonspecifically or with low specificity. Since claims 11 and 19 directly depend from claim 1, both of these claims incorporate the new limitations of claim 1 as well.

The teachings of Dauer and Grevelding have been briefly discussed above. As discussed previously, Dzieglewska teaches a method of isolating nucleic acid from a sample of cells comprising: (a) binding cells in the sample to a solid supports to isolate cells from the sample; (b) lysing the isolated cells to release nucleic acid; and (c) binding nucleic acid released from the lysed cells to the same solid support (abstract; page 4, lines 15-22; emphasis added). Dzieglewska expressly teaches that "[f]ollowing cell binding, the isolated or support-bound cells are lysed to release their nucleic acid" (page 11, lines 14-15) and discloses a wide variety of cell lysis methods on page 11, line 16 through page 13, line 11. Dzieglewska does not teach or suggest a method of nucleic acid amplification that does not comprise lysing the target cell to release the nucleic acid prior to amplification, as required by the presently amended claim 1 and by claims 11 and 19.

Much like Dauer and Grevelding, Dzieglewska does not teach or suggest nonspecific or low-specificity magnetic separation of mammalian cells, particularly leukocytes or epithelial cells. Moreover, much like Dauer and Grevelding, Dzieglewska does not provide any indication that

direct PCR amplification would be effective on mammalian cells, particularly leukocytes or epithelial cells. Since the combination of Dauer, Grevelding and Dzieglewska fails to teach several important elements of claims 11 and 19 as amended herein, the Office has failed to establish a *prima facie* case of obviousness. Accordingly, it is respectfully submitted that this rejection under 35 U.S.C. § 103(a) may properly be withdrawn.

Dauer in View of Grevelding and Further in View of Iinuma

Claim 26 is rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Dauer in view of Grevelding as applied to claims 1-4, 6-9, 12-13, 18, 20-25 and 28 above and further in view of Iinuma *et al.* (*Int. J. Cancer* 2000, 89:337-344, hereinafter "Iinuma").

The Office acknowledges that neither Dauer nor Grevelding teaches that the target cells can comprise leukocytes. To cure this deficiency of Dauer and Grevelding, the Office cites Iinuma, which allegedly teaches that leukocytes can be specifically targeted by magnetic beads comprising antibodies (p. 337, col. 2).

The Office argues that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have analyzed the target cells of Iinuma using the method of separation taught by Dauer to arrive at the claimed invention with a reasonable expectation for success. Iinuma allegedly states: "prepared cells were resuspended in 80 µl of BSA-PBS mixed with 20 µl of CD45 microbeads for 15 min at 4°C and passed down the MACS column" (p. 338, col. 1). Accordingly, the Office concludes that one of ordinary skill in the art at the time the invention was made would have been motivated to have analyzed the target cells of Iinuma using the method of separation taught by Dauer to arrive at the claimed invention with a reasonable expectation for success.

As an initial matter, this rejection is rendered moot with respect to claim 26 by the cancellation of this claim. However, since the limitation of claim 26 has been incorporated to some extent into claims 1 and 38, this rejection will be addressed herein in order to advance prosecution of this application.

The obviousness analysis under 35 U.S.C. § 103(a) requires the consideration of the differences between the claimed invention "as a whole" and the prior art. Thus, here it is critical to understand the difference between the claimed invention "as a whole" and the inventions of the cited references, namely, Dauer, Grevelding and Iinuma, "as a whole".

The teachings of Dauer and Grevelding have been briefly discussed above. As discussed previously, Iinuma teaches highly specific separation of $CD45^+$ cells using magnetic microbeads coated with anti-CD45 antibodies (page 338, emphasis added). Much like Dauer and Grevelding, Iinuma does not teach or suggest nonspecific or low-specificity magnetic separation of mammalian cells, particularly leukocytes or epithelial cells. Moreover, much like Dauer and Grevelding, Iinuma does not provide any indication that direct PCR amplification would be effective on mammalian cells, particularly leukocytes or epithelial cells.

In contrast, the present invention is based on the observation that magnetic beads that do not bind to a target cell with high specificity can nevertheless be used successfully to isolate leukocytes from a whole blood sample or epithelial cells from a saliva sample to such an extent that these cells may be subjected to nucleic acid amplification (e.g., PCR of the HLA-A gene for HLA typing) without having to elute the nucleic acid from the magnetic beads.

Whole blood is a complex mixture of cells (e.g., leukocytes, erythrocytes and platelets), proteins, lipids, carbohydrates and other low-molecular weight compounds, many of which tend to affect the accuracy of analytical methods to various extents. The ratio of leukocytes to erythrocytes, which lack nucleic acids, is approximately 1/1000. Even 1% of whole blood in a sample can effectively inhibit a nucleic acid amplification reaction, which is believed to be due to the binding of heme to DNA polymerase. Thus, isolation of leukocytes from whole blood is critical for nucleic acid amplification. Conventional centrifugation techniques for separating leukocytes are labor-intensive and time consuming. Most commercially available methods at the time of the invention involved the use of CD45 antibody-coated magnetic particles (*see, e.g.*, Iinuma), which are not suitable for routine use due to the high price and biological instability of antibody-coated beads.

The present inventors discovered that leukocytes could be successfully separated from the other components in whole blood by using unmodified magnetic particles or particles modified with organic molecules such as carboxyl, hydroxyl or epoxy groups, even in the absence of specific interactions between the magnetic particles and leukocytes. (*See, e.g.*, Example 1.) As a result, interfering contaminants in the whole blood, such as erythrocytes and proteins, could be largely eliminated. (*See, e.g.*, Fig. 1, lanes 3 and 4.) This discovery went contrary to the common practice of using immunomagnetic particles for the separation of leukocytes from whole blood and could not have been reasonably anticipated based on the disclosure of the cited prior art references. Additionally, it was surprisingly found that the leukocyte-magnetic particle complex could be used as a template for nucleic acid amplification without nucleic acid elution, which allows integrating the separation and amplification steps into a single, easy-to-automate process.

Much like whole blood, saliva is also a complex mixture of cells (e.g., epithelial cells), electrolytes, mucus, antibacterial compounds and various protein enzymes. Because saliva is fairly abundant and convenient to collect, saliva samples have been used for DNA-based diagnostics for a while. However, since saliva contains a number of substances that may interfere with nucleic acid amplification, separation of nucleated cells prior to amplification is desirable. Historically, such separation has been accomplished by centrifugation, which is somewhat difficult to miniaturize. Immunomagnetic separation of epithelial cells from saliva samples has also been used, but it suffers from the same shortcomings as discussed above in the context of leukocytes and whole blood.

The present inventors discovered that epithelial cells could be successfully isolated from the other components in saliva by using unmodified magnetic particles or particles modified with organic molecules such as carboxyl, hydroxyl or epoxy groups, even in the absence of specific interactions between the magnetic particles and epithelial cells. (*See, e.g.*, Example 2.) As a result, interfering components in the saliva could be significantly reduced to the point where they no longer prevent nucleic acid amplification. (*See, e.g.*, Fig. 1, lanes 5 and 6.) Once again, this discovery went against to the common practice of using immunomagnetic particles for the separation of epithelial cells (*see, e.g.*, Hardingham *et al.*, *Cancer Res.*, 53:3455-3458 (1993), which was listed but not relied upon in the Office Action) and could not have been reasonably expected based on the

disclosure of the cited prior art references. Additionally, it was surprisingly found that the epithelial cell-magnetic particle conjugate could be used as a template for nucleic acid amplification without nucleic acid elution, which allows integrating the separation and amplification steps into a single, easy-to-automate process.

Thus, the combination of Dauer, Grevelding and Iinuma fails to teach each and every element of claims 1 and 38 as amended, and there was no adequate motivation to combine the references with a reasonable expectation of success. Accordingly, it is respectfully submitted that this rejection under 35 U.S.C. § 103(a) is not applicable to claims 1 and 38 as amended.

CONCLUSION

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue. If it is determined that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the event the U.S. Patent and Trademark office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket No. 514572000700. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

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